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THE IDENTIFICATION AND DISTRIBUTION OF KEY PROTEOGLYCANS IN CANINE CRUCIATE LIGAMENT

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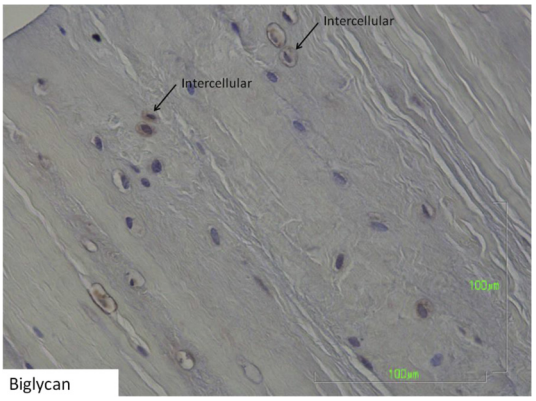
Purpose: Cranial cruciate ligament (CCL) rupture is a common orthopaedic condition in dogs, which can be due to trauma (least common) or by non-contact injury (most common). Rupture of the CCL has serious functional implications, leading to osteoarthritis. The aetopathogenesis of canine cruciate rupture is unknown but changes in CCL extracellular matrix metabolism have been identified in dog breeds with differing risks of ligament rupture. Proteoglycans (PGs) are one of the unique components of tendon and ligament extracellular matrix, consisting of one or more polysaccharide glycosaminoglycan side chains and a unique core protein. Decorin and biglycan, are members of the small leucine rich PGs, that bind to collagen fibrils and organise collagen fibrillogenesis. Aggrecan and versican are two large PGs which maintain tendon and ligament and have a capacity to withstand compressive forces associated with loading. We hypothesise that PGs are key components of the CCL ECM and therefore may have a role in determining risk of CCL rupture in susceptible dog breeds.

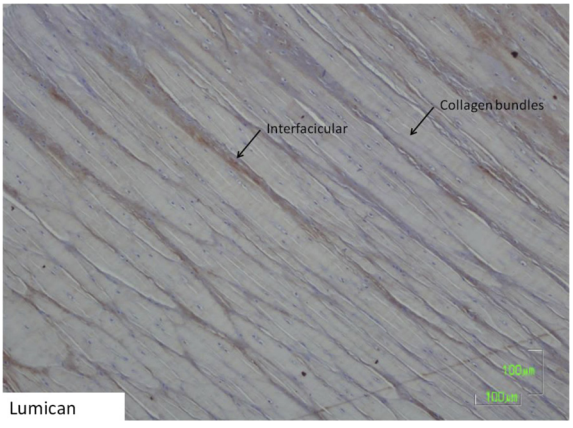
Methods: CCls were harvested from canine crossbreds (n=6 Staffordshire bull terrier cross) with no macroscopic evidence of stifle joint pathology. Each CCL was divided into three sections (origin, middle, and insertion). For western blot analysis, ligament samples were pulverized with a mortar and pestle using liquid nitrogen and proteins were extracted with a 4 M guanidinium chloride extraction buffer. For immunohistochemistry, longitudinal sections were cut to immunolocalise PG-core proteins and glycosaminoglycans side chain using antibodies to chondroitin sulphate stubs, chondroitin-0- sulphate (1-B-5) chondroitin-4-sulphate (2-B-6).

Results: Western blot analysis confirmed that all PGs/glycosaminoglycans were expressed in each region in CCL. Immunohistochemical analysis demonstrated that each proteoglycan had a unique localisation within the CCL. Decorin, lumican, 1-B-5 and 2-B-6 were evident mostly in collagen fibre bundles and interfacicular regions. In contrast, biglycan appeared to be localised to the cells. Versican was immunopositive in all interfacicular, interbundle and cellular regions.

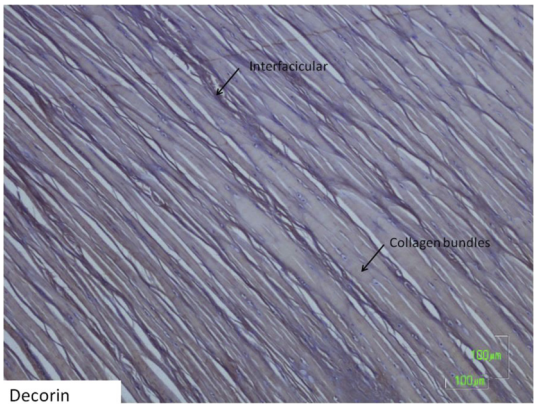
Conclusion: Overall, the distribution of extracellular matrix constituents within the CCL
provides an insight into the regional differences in tissue structure, which are likely to provide essential functionality in the healthy ligament. Future work will include semi-quantitative immunohistochemical analysis of CCL proteoglycans in a low (greyhound) and high (Labrador) risk breeds to ligament degeneration and rupture.



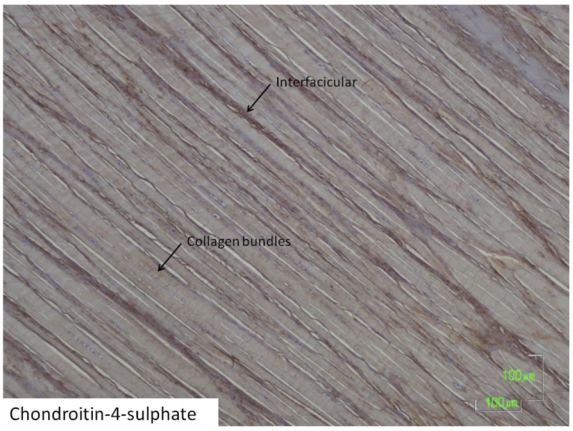
Biglycan



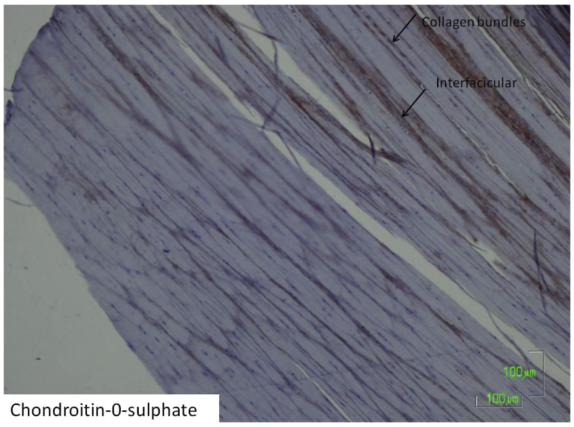
Lumican

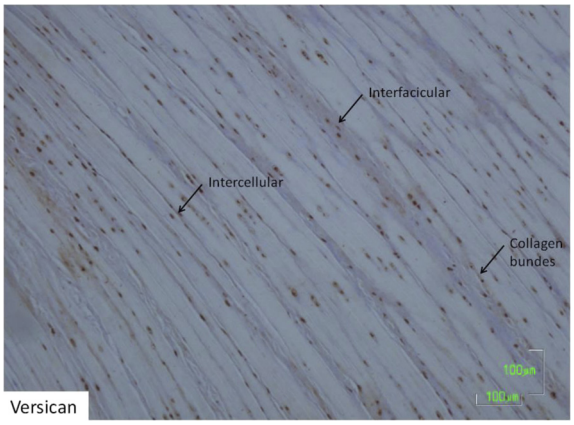


Decorin



Chondroitin-4-sulphate





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IMMEDIATE AND 4-WEEK EFFECTS OF A BRACE ON SYMPTOMS AND FUNCTION IN PEOPLE WITH KNEE OSTEOARTHRITIS AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION: A PILOT STUDY

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Purpose: To investigate the immediate and 4-week effects of a frontal plane unloader knee brace on symptoms and function in people with knee osteoarthritis (OA) after anterior cruciate ligament reconstruction (ACLR).
Methods: Two cohorts were recruited from the community in Melbourne, Australia. Volunteers were eligible if they had undergone a primary ACLR 5-20 years previously, and had radiographic (Kellgren & Lawrence grade $\geq$2) and symptomatic (Knee Injury and Osteoarthritis Outcome Score (KOOS) criteria) knee OA. Eighteen participants took part in the immediate effects study. Symptoms were assessed during hop for distance (HD) and foot-tap (FT) tasks, and function was assessed during HD (total distance hopped), under three test conditions applied in a random order: i) no brace, ii) unadjusted brace (sagittal plane support, no frontal plane adjustment) and iii) unadjusted brace (sagittal plane support with frontal plane adjustment). The direction of frontal plane adjustment (varus/valgus) was selected based on compartmental distribution of OA (i.e. medial or lateral), as well as tibiofemoral alignment on anteroposterior radiograph. Following each task, participants rated their average level of pain, ease of performance, knee confidence, and knee stability on separate visual analogue scales (VAS). Eleven participants in the medium-term study completed the no-brace condition at baseline, and were then randomised to wear either the unadjusted or adjusted brace for four weeks. They repeated the HD and FT tasks at four-week follow-up in both the unadjusted and adjusted brace. Friedman tests evaluated differences between the three brace conditions for each variable ($p<0.05$). Where indicated, post hoc tests (Wilcoxon signed-rank tests) were used to identify differences between conditions, with significance adjusted to $p<0.0167$ for multiple comparisons. The proportion of participants who experienced immediate or four-week changes greater than the minimal clinically important difference for VAS ($\geq$15mm) was calculated. Chi square tests evaluated the effect of allocated brace condition on four-week outcomes ($p<0.05$).
Results: The immediate effects cohort consisted of 12 females (mean±SD age 42±12 body mass index 25±4; KOOS subscales: pain 76±14, symptoms 68±13, activities of daily living 86±9, sport/recreation 59±23, quality of life 45±18). For HD, there was a significant effect of brace condition for ease ($p=0.030$) and confidence ($p<0.001$), and for pain during FT ($p=0.008$). Post hoc tests revealed significant improvements in confidence during HD with the unadjusted and adjusted braces compared to no brace ($p<0.001$). Pain during FT was significantly reduced with the unadjusted brace compared to no brace ($p=0.012$), with trends for significantly reduced pain with the adjusted brace ($p=0.017$). In the four-week cohort (5 females, age 37±7, body mass index 28±4; KOOS subscales: pain 65±14, symptoms 64±16, activities of daily living 82±17, sport/recreation 52±26, quality of life 41±12), significant brace effects were found for confidence ($p=0.013$) and stability ($p=0.000$) during HD, and pain ($p=0.015$), ease ($p=0.024$), confidence ($p=0.006$) and stability ($p=0.001$) during FT. Post hoc tests revealed significant improvements in symptoms for all variables with the adjusted and unadjusted brace compared to no brace ($p<0.0167$), with the exception of pain during FT with the unadjusted brace compared to no brace. No significant differences were observed between the adjusted and unadjusted brace. A greater proportion of participants reported improvements in symptoms $\geq$15mm after four weeks of wear compared to immediate effects, particularly on the FT test. There was no significant effect of allocated brace on four-week outcomes.
Conclusion: In younger individuals with knee OA after ACLR, the unloader brace produces immediate improvements in ease and confidence during HD, and pain during FT. The brace appears to have greater effects when worn for a prolonged period of time, especially during test that resembles daily tasks (FT). Findings suggest that additional interventions such as exercise may be required to improve performance, pain and ease during a higher-level task such as hopping. The lack of differences found between the adjusted and unadjusted brace conditions suggests that frontal plane adjustment may not be necessary to improve symptoms in people with knee OA after ACLR.

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SYNOVIAL MESENCHYMAL STEM CELLS PROMOTE HEALING OF MASSIVE MENISCUS DEFECT AUGMENTED BY ACHILLES TENDON IN A RAT MODEL

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Purpose: We previously reported that transplantation of Achilles tendon treated with BMP-7 promotes meniscus regeneration in a rat massive meniscus defect model. However, there remains the problem about the healing of transplanted tendon and native meniscus or peripheral capsule. Mesenchymal stem cells (MSCs) derived from synovium have a high proliferation and chondrogenic potential. In this study, we investigated whether synovial MSCs promoted meniscus regeneration augmented by tendon transplantation in a rat massive meniscus defect model.
Methods: This study was approved by the Animal Experimentation Committee of our institution. MSC preparation: Synovial MSCs are isolated as previously reported. For the administration, 1 million synovial MSCs were suspended in 30μl PBS.
Animals and surgery: Wild type male Lewis rats at 11-13 weeks were used. Achilles tendon was harvested and placed in the solution suspended with synovial MSCs for 10 minutes before transplantation. After anterior half of medial meniscus was resected, the pretreated tendon was transplanted into the meniscus defect and sutured with joint capsule. Residual MSCs suspended in the solution was also injected into the knee joint after closing the patellar tendon (tendon + MSC group). As controls, same number of rats had transplantation of tendon without MSCs (Tendon group), or only meniscectomy (Control group). Macroscopic and microscopic findings of regenerated meniscus were analyzed. Cartilage degeneration was also analyzed for the tibial plateu.
In vivo bioluminescent imaging (IVIS): To chase the MSCs, IVIS was used to detect photons from synovial MSCs derived from luciferase transgenic rats (Luc^{+} synovial MSCs).